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Flow sandwich-type immunoassay in microfluidic devices based on negative dielectrophoresis

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Abstract

Microparticles have been manipulated in a microfluidic channel by means of negative dielectrophoresis (n-DEP), and the approach applied to a heterogeneous immunoassay system. A microfluidic device, with three-dimensional (3-D) microelectrodes fabricated on two substrates, was used to manipulate particle flow in the channel and to capture the particles in the caged area that was enclosed by the collector electrodes. Polystyrene microparticles (6 μm diameters) modified with anti-mouse immunoglobulin G (IgG) were manipulated and captured in the caged area when surrounded by intense n-DEP electric fields. Specifically, particles were trapped when AC voltages with amplitudes of 6–15 V_{peak} and frequencies over 500 kHz were applied to the two facing microelectrodes. A heterogeneous sandwich immunoassay was achieved by successively injecting a sample solution containing mouse antigen (IgG), and a solution containing a secondary antibody with a signal source (FITC-labeled anti-mouse IgG antibody), into the channel. The fluorescence intensity from captured particles in the caged area increased with increasing concentrations (10 ng/ml to 10 μg/ml) of mouse IgG. The described system enables mouse IgG to be assayed in 40 min. Thus, the automatic separation of free fractions from desired analytes and labeled antibodies can be achieved using a microfluidic device based on n-DEP.

Keywords: Dielectrophoresis; Sandwich immunoassay; Microfluidic device; Separation; Microparticles

1. Introduction

Microfluidic technology is widely used for immunoassays in order to improve analytical performance characteristics, such as rapid analysis time, reliable and sensitive detection, easy handling and low consumption of reagents (Bange et al., 2005). In heterogeneous sandwich immunoassays with continuous flow systems, a primary antibody is attached to the channel walls, thus permitting the separation of undesired fractions from both desired analytes and from labeled antibodies used as a signal source (Lim et al., 2003). Devices with microfluidic channels have a higher surface area to volume ratio for antibody immobilization compared to conventional immunoassays using microtiter plates.

Microparticles have also been used as solid supports for the modification with primary antibodies. They can dramatically increase the surface area and, therefore, increase the efficiency of the immunoreaction between the immobilized antibody and the antigen present in a continuously-flowing solution. To make immunoassays with microparticles reliable and practical, it is necessary to develop technologies for manipulating the particles in the channel. Kitamori and coworkers fabricated a 'dam' structure for retaining polystyrene microparticles in a glass-based microchannel (Sato et al., 2000, 2002). The possibility of conducting immunoassays on a fluidic microchip using incorporated microparticles was investigated by using thermal lens microscopy. Magnetic beads were also used for immunoprotein support and separation, since these beads can be easily manipulated in the channel by applying a magnetic field (Choi et al., 2002; Wijayawardhana et al., 1999; Thomas et al., 2004). When the applied field is turned off, the magnetic beads are released into the waste chamber, permitting reuse of the fluidic device for another assay.

Microparticles can also be manipulated in a flowing stream by dielectrophoresis (DEP). DEP is an electrokinetic phenomenon produced by the interaction of an induced polarization with a spatially inhomogeneous electric field. DEP has been used to control micro- and nano-objects. Currently, DEP is attractive

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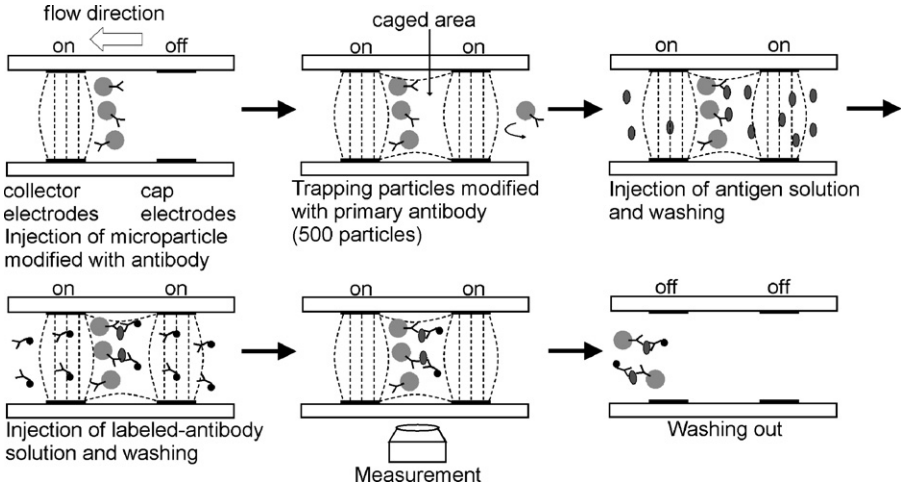

Fig. 1. Principle behind the assay methodology, combining the microfluidic channel and the n-DEP-based manipulation techniques.

for sorting and separating particles and cells of interest because the DEP force is created using simple electrode components, without any moving actuators. Particle manipulation with 3-D microelectrode systems has been combined with fluidic channels (Fiedler et al., 1998; Muller et al., 1999; Schnelle et al., 1999a,b; Durr et al., 2003) and applied to lateral handling and trapping of live cells (Seger et al., 2004; Hu et al., 2005). In addition, positive DEP (p-DEP) (Gray et al., 2004; Matsumoto et al., 1994; Masuda et al., 1989; Ho et al., 2006; Taff and Voldman, 2005) and negative DEP (n-DEP) (Suzuki et al., 2004; Matsue et al., 1997) have been applied to patterning microparticles and biological cells. The single-particle manipulation (Ogata et al., 2001; Schnelle et al., 1999c; Matsue et al., 1993) has also been achieved. The assembly of particles onto micropatterned electrodes based on DEP has been utilized to detect the immunoreaction (Velve and Kaler, 1999; Auerswald et al., 2005).

We report here the development and characterization of an integrated microfluidic system for immunoassays using antibody-modified polystyrene particles. The system has a caged area surrounded by n-DEP-generated electric barriers that capture microparticles modified with anti-mouse IgG. Fig. 1 shows the principle behind the assay. A fixed amount of flowing particles with immobilized antibody are trapped in the caged area created by n-DEP. Then, the sample solution containing antigen (mouse IgG), and the solution containing anti-mouse IgG antibody conjugated with fluorescein isothiocianate (FITC), are successively injected into the channel that automatically separates any unreacted molecules. The fluorescence intensity of the particles in the caged area is measured by fluorescence microscopy. This fluidic device is reusable since the particles are easily removed from the caged area by switching off the electric fields and collecting the particles in the waste reservoir.

2. Theory

Particles in a suspended solution are polarized when an alternating electrical field is applied. DEP refers to the migration of a particle resulting from the interaction between the induced polarization and the spatially inhomogeneous electric field (Pohl, 1978; Jones, 1995; Morgan and Green, 2002). The time-averaged DEP force, $\langle \overline{F}_{\mathrm{DEP}} \rangle$ [N], acting on a homogeneous dielectric particle suspended in a dielectric fluid medium, is given by

$$\langle \overline{F}_{\mathrm{DEP}} \rangle = 2\pi\varepsilon_{\mathrm{m}} a^3 Re[\underline{K}(\omega)]\nabla E_{\mathrm{rms}}^2 \quad (1)$$

where a is the particle radius [m], ε_{m} is the permittivity of the suspension medium [F/m], E_{rms} is the root-mean-square (rms) electric field [V/m], ∇ is the del vector operator, and $\mathrm{Re}[\underline{K}(\omega)]$ is the real part of the Clausius-Mossotti factor, given by

$$\underline{K}(\omega) = \frac{\underline{\varepsilon_{\mathrm{p}}} - \underline{\varepsilon_{\mathrm{m}}}}{\underline{\varepsilon_{\mathrm{p}}} + 2\underline{\varepsilon_{\mathrm{m}}}} \quad (2)$$

In this equation, $\underline{\varepsilon_{\mathrm{m}}}$ and $\underline{\varepsilon_{\mathrm{p}}}$ are the complex permittivity of the medium and particle, respectively, and

$$\underline{\varepsilon} = \varepsilon - \frac{\sigma}{\omega} j \quad (3)$$

where σ is the conductivity [S/m], ε is the permittivity, ω is the angular frequency ($= 2\pi f$, where f is the applied frequency [Hz]) and $j = \sqrt{-1}$. The direction and magnitude of the DEP force acting on the particles depends on the dielectric properties of the particles, the suspension solution, the electrode configurations, and the voltage and frequency of the applied electric field. Particles undergoing positive DEP migrate toward the highest electric field following electric field intensity gradients, while particles undergoing negative DEP migrate in the opposite direction.

Microfluidic devices with parallel line electrodes on the top and bottom of the assembly have been developed and studied by Fuhr's group (Fiedler et al., 1998; Muller et al., 1999; Schnelle et al., 1999a,b). Electric fields with frequencies compatible with n-DEP cause the particles to be repelled from the electric barrier formed by the two electrodes. The maximum DEP force, F_{DEP} [N], which the particles experience half-way between the electrodes, has been calculated (Schnelle et al., 1999a,b):

$$F_{\mathrm{DEP}} = \frac{27}{32}\pi^2 \varepsilon_{\mathrm{m}} \left(\frac{a}{h}\right)^3 Re[\underline{K}(\omega)] V_{\mathrm{rms}}^2 \quad (4)$$